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Title of Invention	Material for Separating Components in A Biologic Fluid

Application Number :

Date :

First Named Applicant: Marcus STORR

Attorney Docket Number: PN0282-US01

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Description

Material for Separating Components in A Biologic Fluid

Background of Invention

[0001] The present invention relates to a separating material, a novel method for producing the same, and the use of the separating material in several separating applications, particularly in separating components of biologic fluids.

[0002] By "separating material" is meant a material that is useful as an adsorption material and/or as a dialysis material and/or as a filtration material for the separation from a fluid of substances that have specific chemical or physical properties or that are recognized by specific recognition compounds, especially separation of substances from a liquid. Separating materials in the sense of the present invention is particularly useful in the separation or depletion of undesirable substances from liquids, e.g. the adsorptive separation of toxins from blood plasma.

[0003] Separating materials for adsorptive separation applica-

tions generally comprise a solid phase substrate material or matrix material which carries on its surface active sites for the more or less specific adsorption of compounds having particular properties, e.g. positive or negative charges, specific chemical structures or functional groups etc. The solid phase substrate material may often be a porous or non-porous polymer having functional surface groups or chains of a graft copolymer being functionalized and being formed by graft polymerization of monomers onto the surface of the polymeric matrix material.

[0004] The US Patent No. 5,556,708 describes a method for the production of an adsorption material by graft polymerization of a nitrogen-containing polymer with an ethylenically unsaturated monomer in an aqueous environment in the presence of two reactants, the two reactants consisting of carbon tetrachloride and a reducing agent, selected from sodium dithionite, rongalite, hydrazine, and ascorbic acid. According to the description and the examples of US Patent No. 5,556,708, sodium dithionite seems to be the only one reducing agent that had been tested. Also, even though it is claimed that the nitrogen-containing polymer may be selected from polyamides, polysulfonamides,

polyurethanes, and polymers having primary and secondary amine groups in a side chain, only a polyamide membrane, particularly a nylon 6,6 membrane, had been tested in the examples as the nitrogen-containing polymer. US Patent No. 5,556,708 leaves unclear whether and how the method may work with a nitrogen-containing polymer having primary and secondary amine groups. It is known that amides form radicals with the reducing agents used according to US Patent No. 5,556,708, but there is no mechanistical explanation of how the graft polymerization should work with primary and secondary amines.

[0005] One major disadvantage of the method of US Patent No. 5,556708 is the prescribed use of an organic reactant, especially of carbon tetrachloride in the graft polymerization process. Even if the produced material is thoroughly cleaned after the production process, there will still be amounts of carbon tetrachloride remaining in the porous polymeric structure. The toxicity of carbon tetrachloride thus makes the produced adsorption material inappropriate for medical applications, such as the adsorption of toxins from blood or in hemodialysis. On the other hand, the substantially complete removal of carbon tetrachloride from the adsorption material of US Patent No. 5,556,708

by exhaustive rinsing or washing of the material would cause enormous costs and would make the material commercially unattractive.

[0006] Summarizing, the disadvantages of prior art separating materials include the following: the substrate materials are not biocompatible or blood compatible, thus the materials are not useful for medical applications; the reactions to produce such separating materials require organic solvents which are toxic or biohazardous, thus the materials are not useful for medical applications; the reaction conditions to produce such separating materials are often harsh, so that the preparation methods are restricted to reactants which withstand such conditions; and if UV activation is used, the reactions to produce such separating materials, do not provide for a uniform functionalization over the entire surface of a porous polymeric matrix.

[0007] It is an object of the present invention to provide a separation material and a method for producing the same, whereby the above-mentioned disadvantages of the prior art are overcome. It is another object of the present invention to provide a separation material that is useful for medical applications.

SUMMARY OF INVENTION

[0008] Accordingly, the present invention provides a separating material producible by:

[0009] a)providing a solid substrate, having amino-functional groups coupled to the substrate surface,

[0010] b)covalently coupling of the amino-functional groups with a thermally labile radical initiator, and

[0011] c)contacting the substrate surface with a solution of polymerizable monomers under conditions where thermally initiated graft copolymerization of the monomers takes place, to form a structure of adjacent functional polymer chains on the surface of the substrate.

[0012] In another aspect the present invention provides a method for the production of a separating material by:

[0013] a)providing a solid substrate, having amino-functional groups coupled to the substrate surface,

[0014] b)covalently coupling of the amino-functional groups with a thermally labile radical initiator, and

[0015] c)contacting the substrate surface with a solution of polymerizable monomers under conditions where thermally initiated graft copolymerization of themonomers takes place, to form a structure of adjacent functional polymer chains on the surface of the substrate.

[0016] Preferably, the separating material of the present inven-

tion is produced by the afore-mentioned method. One advantage of the present invention is that the method of producing the separating material of the present invention does not require an organic solvent, such as carbon tetrachloride, which is difficult to remove from the final product, and which may be toxic or at least harmful to a patient, when the separating material is used in medical applications and extracorporeally contacted to any liquid or body fluid that is introduced or reintroduced into the patient's body.

[0017] Another advantage of the present invention lies in the covalently coupling of the radical initiator to the amino-functional groups on the solid substrate. Thereby, the occurrence of homopolymerization in the reaction solution is avoided or at least minimized. The radical initiator, which is bound to the solid substrate, forms radicals upon temperature increase, and part of the radical initiator structure becomes part of the polymer chains, which are formed from the solid substrate surface. The polymer chains of the present invention develop from the surface of the substrate without the formation of undesired cross-linkages between the chains, thus the process of the present invention is considered to provide a very "clean"

chemistry.

[0018] Another advantage of the present invention is based on the use of thermally labile radical initiators, which can be chosen to ensure mild reaction conditions and to avoid additional reactants which may react with the substrate or the monomers in an undesired manner. The temperatures to initiate radical formation of useful radical initiators typically lie within the range of 50°C to 120°C, preferably in the range of 70°C to 100°C. A useful temperature range of the polymerization reaction is from the 10 hour half-life temperature of the radical initiator to about 20 to 25 degrees above that 10 hour half-life temperature. By adjusting the reaction temperature it is further possible to very precisely control the polymerization reaction, e.g. onset of the reaction, reaction speed, degree of polymerization, etc.

[0019] In a preferred embodiment of the separating material of the present invention, the solid substrate is a porous polymeric material. The porosity of the substrate material provides a large surface area for the contact between the separating material and the fluid.

[0020] An advantageous use of the separating material is the medical application in the extracorporeal treatment of hu-

man or animal blood or other body fluids, e.g. hemodialysis, filtration, and/or removal of undesired substances from the blood by adsorption of such substances to the separating material. Usually, in such applications the blood of a patient is extracorporeally separated into the blood cells and the blood plasma (or blood serum), the latter containing most of the substances to be removed by the treatment. In another preferred embodiment of the invention the porous polymeric material has a pore size that is sufficiently large to allow passage of blood plasma, or blood serum through the substrate material. This allows the blood plasma or blood serum to get in contact with the entire surface area within the pores of the separating material. In another embodiment the porous polymeric material has a pore size that is sufficiently large to allow passage of blood plasma, or blood serum through the substrate material, whereby the pore size is sufficiently small to avoid passage of the blood cells. This allows the use of the separation material, if it is in the form of a membrane or a hollow fiber membrane, to separate the blood cells from the blood plasma by passing whole blood onto or by the membrane. Thereby, the blood cells are retained on one side of the membrane, whereas blood

plasma can pass through the pores of the membrane to the opposite side of the membrane. Thus, blood cells are filtered from the blood plasma. While the blood plasma is passing the separating material membrane, it contacts the active surface of the material within the pores of the material. Thereby, the separating material deletes the blood plasma from undesired substances by adsorption. Afterwards, the depleted or purified blood plasma may be recombined with the blood cells and, for example, be reinjected into the patient's circulation, or it may be stored for later use.

[0021] The separating material of the present invention may be provided in any form, but preferably is in the form of a membrane, a hollow fiber membrane, a particle bed, a fiber mat, or beads. Most preferably, it is in the form of a hollow fiber membrane, as is well known from hemodialysis applications. Multiple hollow fiber membranes can by known procedures be potted into tubes, and the tubes can be fitted with ports in a known manner to provide separating units, which preferably are in the form of cartridges to be inserted into dialysis apparatuses. If the separating material of the present invention is provided in the form of beads, such beads can, for example, be packed into

columns for the passage of the treated fluid, e.g., blood plasma.

[0022] In another preferred embodiment, the separating material of the present invention is made of a biocompatible material, to avoid any hazardous effects on a treated body liquid of a patient or on the patient herself, if the treated liquid is reinfused into the patient.

[0023] Preferred materials useful for the preparation of the separating material of the present invention are selected from the group consisting of polyacrylates, polystyrene, polyethylene oxide, cellulose, cellulose derivatives, polyethersulfone (PES), polypropylene (PP), polysulfone (PSU), polymethylmethacrylate (PMMA), polycarbonate (PC), polyacrylonitrile (PAN), polyamide (PA), polytetrafluorethylene (PTFE), cellulose acetate (CA), regenerated cellulose, and blends or copolymers of the foregoing, or blends or copolymers with hydrophilizing polymers, preferably with polyvinyipyrollidone (PVP) or polyethyleneoxide (PEO).

[0024] Preferably, the amino-functional groups on the solid substrate for the production of the separating material of the invention are primary amino groups, though secondary amino groups may also be useful. Primary amino groups

provide for a higher reactivity.

[0025] In a highly preferred embodiment of the present invention, the thermally labile radical initiator, as the starting material before coupling to the amine groups on the solid substrate, comprises at least one, preferably two, carboxylic groups. In the reaction of coupling of the radical initiator to the amine group of the substrate, the carboxylic groups are preferably activated by a water soluble carbodiimide, for example 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDAC) whichforms active o-acylurea intermediates. After initial activation, the carboxyl groups will react with, e.g., N-hydroxysuccinimide (NHS) to form an active ester, which couples with the primary amino groups on the surface of the substrate.

[0026] Useful thermally labile radical initiators include compounds which decompose to give free radicals on thermal activation. Preferably, the thermally labile radical initiator being selected among azo compounds or peroxides. Most preferred radical initiators are 4,4'-azobis-(4-cyanovaleric acid) or 2,2'-azobis-[N-(2-carboxyethyl)-2-methylpropicnamidine .The monomers useful to form the polymer chains from

the substrate surface by graft polymerization are selected from compounds having a polymerizable double bond. Preferred monomers can be divided into three groups: (1) monomers providing positive or negative charges, (2) monomers for binding affinity ligands, and (3) inert hemocompatible monomers.

[0027] Preferred monomers of the first group (1) are selected from N,N-Dimethylaminoethyl acrylamide, N,N-Diethylaminoethyl acrylamide, N,N-Dimethylaminopropyl acrylamide (DMPA), N,N-Dimethylaminopropyl methacrylamide, N,N-Dimethylaminoethyl methacrylate, N,N-Diethylaminoethyl methacrylate, N,N-Dimethylaminoethyl acrylate, N-Morpholinoethyl acrylate, N-Morpholinoethylmethacrylate, 1-Vinylimidazole, Trimethylammoniumethyl acrylamide, Trimethylammoniumpropyl methacrylamide, and Trimethylammoniumethyl methacrylate. The most preferred monomer of this group is Dimethylaminopropyl acrylamide (DMPA).

[0028] Preferred monomers of the second group (2) are selected from Glycidyl acrylate, Glycidyl methacrylate, Vinyl glycidyl ether, and Vinyl glycidyl urethane. The most preferred

monomer of this group is Glycidyl methacrylate.

[0029] Preferred monomers of the third group (3) are selected from 2-Hydroxyethyl methacrylate, 2-Hydroxypropyl methacrylate, Hydroxymethyl methacrylate, N-Vinylpyrrolidone, 2-Vinyl pyridine, 4-Vinyl pyridine, and N-Vinyl-2-methylimidazole. The most preferred monomer of this group is 2-Hydroxyethyl methacrylate.

[0030] The polymerization reaction can comprise one single type of monomer of the above-mentioned groups, or it can be carried out using two or more different types of monomers from the same group or from different groups.

[0031] Highly preferred polymerizable monomers are selected from compounds of the following formula:

[0032] $H_2C=C(R^1)-C(O)-X-R^2-N(R^3)_2$,

[0033] wherein R^1= hydrogen, methyl or ethyl group; R^2= Cl-C6-alkyl or aryl group; R^3= methyl or ethyl group; and X= NH or O.

[0034] The invention will now be described and further illustrated by way of preferred reaction schemes, examples and the accompanying figures.

BRIEF DESCRIPTION OF DRAWINGS

[0035] Figure 1 illustrates a first reaction scheme, according to

the present invention.

[0036] Figure 2 illustrates a second reaction scheme, according to the present invention.

[0037] Figure 3 illustrates a third reaction scheme, according to the present invention.

[0038] Figure 4 illustrates data of the measurement of the endotoxin concentrations according to example 9, described below.

[0039] Figure 5 illustrates the experimental set-up for dynamic endotoxin adsorption of grafted membranes from citrate-coagulated human blood according to example 10, described below.

[0040] Figure 6 illustrates data of the measurement of the endotoxin concentrations in filtrates according to example 10, described below.

[0041] Figure 7 illustrates data of the measurement of the endotoxin concentrations in the blood reservoir according to example 10, described below.

DETAILED DESCRIPTION

[0042] 1. By way of example the separating material of the present invention can be produced using N,N-dimethylaminopropylacrylamide as the polymerizable monomer. This monomer provides a basic group which is

positively charged at physiologic pH. Therefore, the produced separating material is effective to adsorb negatively charged substances by charge interaction, e.g., bacterial toxins such as endotoxins from gram-negative bacteria, lipoteichoic acid from gram-positive bacteria or bacterial DNA. Using the produced separating material, a number of tests have been carried out with respect to endotoxin removal from plasma or blood (see below). The production of the thus produced separating material is illustrated in reaction scheme 1 shown in Fig. 1.

[0043] In the first reaction step, the polymerization initiator is covalently coupled to the support. Therefore, the amino-group containing supports is reacted with activated esters, e.g., carbodiimide or anhydride activated carboxylic groups of the initiator. Thereby the polymerization initiator is bound to the activated sites. Suitable polymerization initiators are compounds which decompose to give free radicals at thermal activation, e.g. azo compounds or peroxides, and which further carry reactive substituents, e.g. carboxylic groups. Particularly preferred initiators are azo carboxyl compounds, such as 4,4'-azobis(4-cyanovaleric acid) or 2,2'-azobis[N-(2-carboxyethyl)-2-methyl propionamidine]. The carboxyl groups are preferably activated

by the water soluble carbodiimide 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDAC) which forms active o-acylurea intermediates. After initial activation by EDAC, the carboxyl groups will react with N-hydroxysuccinimide (NHS) to form an active ester, which couples with the primary amino groups on the surface of the substrate. When using 4,4'-azobis(4-cyanovaleric acid) as initiator the reaction can be carried out in organic solutions such as DMF, DMSO or toluene. The reaction can also be carried out in aqueous solution at a pH > 12, which is preferable for medical applications.

[0044] Concentration ranges of compounds for modification of 1 - 10 g substrate (membrane or beads) are:

[0045] 20 - 200 ml solvent

[0046] 0.1- 10 g 4,4'-Azobis(4-cyanovaleric acid)

[0047] 0.2 - 20 g EDA

[0048] 0.3 - 20 g NHS

[0049] Reaction temperature: 0 - 30°C, most preferably room temperature for simplicity reasons

[0050] Reaction time: 1 to a several hours

[0051] In the second reaction step the initiator immobilized sur-

face is contacted with a solution of N,N-dimethylaminopropyl acrylamide. The reaction can be carried out in degassed water in an inert atmosphere. The temperature is chosen above the 10 hour half-life temperature of the initiator. The grafting reaction typically uses 1 to 20 g N,N-dimethylaminopropyl acrylamide dissolved in from 10 to 300 ml water. The reaction is typically carried out at from 70 to 95°C, and typically takes from 30 min to several hours.

[0052] Other monomers suitable for introducing positive charges are, inter alia, N,N-dimethylaminoethyl acrylamide, N,N-diethylaminoethyl acrylamide, N,N-dimethylaminoethyl melate N,N-dimethylaminoethyl acrylamide, N,N-dimethylaminopropyl methacrylannide and trimethylammoniumethyl acrylamide.

[0053] 2. In another illustrating example, the separating material of the present invention can be produced using glycidyl methacrylate as the polymerizable monomer. This type of monomer is used for subsequent binding of affinity ligands such as proteins, peptides, antibodies or other biological molecules. The epoxide groups can also be reacted with amino compounds, such as diethylamine, triethylamine or arginine, to give positively charged adsorbents.

Other monomers which comprise both a polymerizable double bond and an oxirane ring are for example glycidyl acrylate, vinyl glycidyl ether and vinyl glycidyl urethane. The production of the thus produced separating material is illustrated in reaction scheme 2 shown in Fig. 2.

[0054] In the first reaction step coupling of polymerization initiator is performed as described above in connection with reaction scheme 1. In the second reaction step the surface is contacted again with a solution of the monomer at elevated temperature in an inert atmosphere.

[0055] Reaction components:

[0056] 1- 10 g Substrate (membrane or beads)

[0057] 1- 20 g Glycidyl methacrylate

[0058] 10 - 400 ml solvents (water, ethanol, ethanol, toluene, DMF, DMSO)

[0059] 3. In another example, the separating material of the present invention can be produced using a mixture of a functional monomer and an inert monomer as the polymerizable monorners. The monomers can be used alone, as also illustrated above, or in a mixture with inert monomers, e.g. vinyl pyrrolidone, hydroxymethyl methacrylamide or hydroxyethyl acrylate in order to in-

crease the hydrophilicity of the polymer or/and to improve the biocompatibility of the materials. The production of the thus produced separating material is illustrated in reaction scheme 3 shown in Fig. 3.

[0060] Example 1 Coupling of 4,4'-azobis(4-cyanovaleric acid) onto macroporous acrylic beads

[0061] 200 g oxirane acrylic resin beads (e.g. Toyo Pearl HW70EC, Tosoh Corp.) having an average epoxy group content of 4.0 mmol/g was aminated with 150 ml conc. ammonia solution (32 wt%) for 3 hours at 40°C. After washing with distilled water, 45 g beads were resuspended in 400 ml DNIF and 10 g 4,4'-azobis(4-cyanovaleric acid). 15 g EDAC and 15 g NHS were added. The batch is agitated for 12 hours at room temperature and afterwards rinsed with water.

[0062] Example 2 Coupling of 4,4'-azobis(4-cyanovaleric acid) onto microporous hollow fiber membranes

[0063] A bundle of polyethersulfon/polyvinylpyrrolidone hollow fiber membranes (144 fibers, 25 cm long, inner diameter 260 µm, outer diameter 340 µm, mean pore diameter 0.3 µm, functionalized with 11 µmol/g primary amino groups by plasma treatment as described in Swedish patent application 020107-8, incorporated herein by reference)

were incubated with 0.6 g 4,4'-azobis(4-cyanovaleric acid) and 0.85 g NHS in 45 ml 0.1 M NaOH. Then 0.85 g EDAC dissolved in 5 ml 0.1 M NaOH was added and agitated for 12 h at room temperature. Afterwards the excess reagents were removed by washing repeatedly with water.

[0064] Example 3 Graft polymerisation of beads with N,N-dimethylaminopropylacrylamide

[0065] 15 g beads prepared as in Example 1 were reacted in a reaction solution of 0.45 g N,N-dimethylaminopropylacrylamide in 75 ml degassed water in a three-necked flask. The reaction was performed with gentle stirring at 75for 3 hours in an atmosphere of nitrogen. The prepared beads were then rinsed as follows: 1 l hot water, 50 ml 1 M NaOH, 50 ml 1 M HCl, 0.5 l wa0.5 l PBS buffer (pH=7.4), 0.5 l 1 M NaCl solution and 0.5 l water.

[0066] Example 4 Graft polymerization of beads with glycidyl methacrylate

[0067] 20 g beads prepared as in Example 1 were reacted in a reaction solution of 8.0 g glycidyl methacrylate in 120 ml toluene in a three-necked flask. The reaction was performed with gentle stirring at 75°C for 3 hours in an atmosphere of nitrogen (reflux condenser). The prepared

beads were then thoroughly rinsed as described in example 3 and dried overnight at 40°C in a vacuum drying oven. The degree of grafting was found to be 125%.

[0068] Example 5 Graft polymerize acrylamide of microporous membranes with N,N-dimethylaminopropyl acrylamide

[0069] A bundle of membranes prepared as in Example 2 was reacted in a reaction solution of 2.5 g N,N-dimethylaminopropyl acrylamide in 40 ml degassed water in a three-necked flask. The reaction was performed with gentle stirring at 75°C for 12 hours in an atmosphere of nitrogen. The prepared membranes were then thoroughly rinsed as described in example 3. To prepare a membrane device the bundle was dried and potted at each end of a 10 mm-diameter poly(carbonate) tube fitted with two ports in the shell.

[0070] Example 6 Graft polymerization of microporous membranes with glycidyl methacrylate

[0071] A bundle of membranes prepared as in Example 2 was reacted in a reaction solution of 1.0 g glycidyl methacrylate in 40 ml Isopropanol water in a three-necked flask. The reaction was performed with gentle stirring at 75°C for 3 hours in an atmosphere of nitrogen. The prepared membranes were then thoroughly rinsed as described in exam-

ple 3 and reacted with 2.0 g oligo arginine in 40 g water, which had been synthesized as described in WO0123413, incorporated herein by reference.

[0072] Example 7 Graft polymerisation of microporous membranes with a mixture of glycidyl methacrylate and hydroxymethyl methacrylamide

[0073] A bundle of membranes prepared as in Example 2 was reacted in a reaction solution of 0.6 g glycidyl methacrylate and 2.4 g hydroxymethyl methacrylamide in 40 ml degassed water in a three-necked flask. The reaction was performed with gentle stirring at 75°C for 3 hours in an atmosphere of nitrogen. The prepared membranes were then thoroughly rinsed as described in example 3 and dried overnight at 40°C in a vacuum drying oven. The degree of grafting is found to be 122.5%.

[0074] Example 8 Determination of the grafting yield and protein binding capacity

[0075] The dynamic protein binding capacity of the adsorbents produced in examples 3, 5, and 6 was determined by establishing the breakthrough curves of the membrane modules or fixed bed columns filled with the grafted beads in dead-end filtration mode and single-pass perfusion mode, respectively. Thereby a solution of bovine

serum albumin (1g/l in 20 mM Tris pH 8.0) was pumped through the modules or columns at a perfusion rate of 1 ml/min. The effluent was monitored by a flow-through UV-detector cell at 280 nm. The results are shown in the following table 1.

Table 1

Adsorbent	Degree of grafting	Dynamic BSA binding capacity [mg/g substrate]
Example 3	117	180
Example 5	104	166
Example 6	108	99

[0076] Example 9 Dynamic endotoxin adsorption of grafted beads from citrate-anticoagulated human blood

[0077] 4 g beads grafted with N,N-dimethylaminopropylacrylamide, as described in example 3, were packed into a poly(carbonate) column. A column with 4 g acrylate beads not reacted with any ligand was used as control. To eliminate potential contamination the column was perfused with 100 ml 30% ethanol (0.1 M NaOH, 8.8 gh NaCl), followed by 200 ml Ringer/ ACD solution and 100 ml pyrogen-free 0.9% saline. Endotoxin (LPS from E coli, 055B.5) was added to freshly donated citrate anticoagulated human blood at a concentra-

tion of 10 EU/ml. 150 ml blood prepared in this manner was then passed through the columns at a flow rate of 1.3 ml/min. Aliquots of 1 ml were taken before and after the test columns and assayed for LPS content using chromogenic Limulus Amebocyte Lysate (LAL) test (Charles River Endosafe, Inc.) as described by K. Duner, (1993) Journal of Biochem. and Biophys. Method26:131-142. The results are shown in Fig. 4.

[0078] Example 10 Dynamic Endetoxin adsorption of grafted membranes, from citrate-anticoagulated human blood

[0079] Membrane modules prepared as in Example 5 were sterilized with steam and rinsed in filtration mode with 200 ml pyrogen-free 0.9% saline. Then 90 ml Citrate-anticoagulated fresh human whole blood spiked with 3 EU/ml Endotoxin (LPS from E.coli, 055B.5) was perfused from a blood reservoir through the module under recirculating conditions, as it is shown in Fig. 5. The blood flow rate was 8 ml/min and plasma is filtrated at a flow rate of 1 ml/min through the membrane of the membrane module. To avoid a dilution effect the first 20 ml blood were withdrawn after perfusion. After 30 min, 90 min, 150 min, 210 min, and 270 min aliquots of 1 ml were taken from the filtrate and from the blood reservoir, as illustrated in Fig.

5 (filtrate sample port; blood sample port), and assayed for LPS content using the LAL test described in example 9. A membrane module with membranes not modified with ligands was used in a control experiment. As shown in Fig. 6 the spiked endotoxin was completely removed from the plasma fraction filtrated through the membrane. As shown in Fig. 7, the treatment resulted in a 100% reduction of endotoxin in the blood pool after 210 minutes of perfusion.

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Claims

[c1] 1.A separating material producible by:
providing a solid substrate, having amino-functional groups coupled to the substrate surface,
covalently coupling of the amino-functional groups with a thermally labile radical initiator,
contacting the substrate surface with a solution of polymerizable monomers under conditions, where thermally initiated graft copolymerization of the monomers takes place, to form a structure of adjacent functional polymer chains on the surface of the substrate.

[c2] 2. The separating material of claim 1, wherein the solid substrate is a porous polymeric material, preferably a porous polymeric material having a pore size that is sufficiently large to allow passage of blood, blood plasma, or blood serum through the substrate material.

[c3] 3. The separating material of any of claims 1 or 2, wherein the solid substrate is in the form of a membrane, a hollow fiber membrane, a particle bed, a fiber mat, or beads, preferably a hollow fiber membrane.

[c4] 4. The separating material of any of claims 1 to 3,

wherein the solid substrate is made of a biocompatible material.

[c5] OLE_LINK45. The separating material of any of claims 1 to 4, wherein the solid substrate is made of a material selected from the group, consisting of polyacrylates, polystyrene, polyethylene oxide, cellulose, cellulose derivatives, polyethersulfone (PES), polypropylene (PP), polysulfone (PSU), poly methyl methacryiate (PMMA), polycarbonate (PC), polyacrylonitrile (PAN), polyamide (PA), polytetrafluorethyiene (PTFE), cellulose acetate (CA), regenerated cellulose, and blends or copolymers of the foregoing, or blends or copolymers with hydrophilizing polymers, preferably with polyvinylpyrollidone (PVP) or polyethyleneoxide (PEO).

[c6] 6. The separating material of any of claims 1 to 5, wherein the amino-functional groups are primary amino groups.

[c7] 7. The separating material of any of claims 1 to 6, wherein the thermally labile radical initiator as the starting material before coupling to the amine groups on, the substrate comprises at least one, preferably two carboxylic groups.

[c8] 8. The separating material of any of claims 1 to 7,

wherein the thermally labile radical initiator includes compounds which decompose to give free radicals on thermal activation, preferably the thermally labile radical initiator being selected among azo compounds or peroxides.

[c9] 9. The separating material of any of claims 1 to 8, wherein the thermally labile radical initiator is 4,4'-azobis-(4-cyanovaleric acid) or 2,2'-azobis-[N-(2-carboxyethyl)-2-methylpropionamidine.

[c10] 10. The separating material of any of claims 1 to 9, wherein the polymerizable monomers are selected from compounds having a polymerizable double bond.

[c11] 11. The separating material of any of claims 1 to 10, wherein the polymerizable monomers are selected from the group, consisting of
acrylic acid, methacrylic acid, vinyl compounds, and derivatives of the foregoing compounds,
N,N-Dimethylaminoethyl acrylamide,
N,N-Diethylaminoethyl acrylamide,
N,N-Dimethylaminopropyl acrylamide (DMPA),
N,N-Dimethylaminopropyl methacrylamide,
N,N-Dimethylaminoethyl methacrylate,
N,N-Diethylaminoethyl methacrylate,

N,N-Dimethylaminoethyl acrylate, N-Morpholinoethyl acrylate, N-Morpholinoethyl methacrylate, 1-Vinylimidazole, Trimethylammoniumethyl acrylamide, Trimethylammoniumpropyl methacrylamide, Trimethylammoniumethyl methacrylate, Glycidyl acrylate, Glycidyl methacrylate, Vinyl glycidyl ether, Vinyl glycidyl rethane, 2-Hydroxyethyl methacrylate, 2-Hydroxypropyl methacrylate, Hydroxymethyl methacrylate, N-Vinylpyrrolidone, 2-Vinyl pyridine, 4-Vinyl pyridine, N-Vinyl-2-methylimidazole.

[c12] 12. The separating material of any of claims 1 to 11, wherein the polymerizable monomers comprise Dimethylaminopropyl acrylamide (DMPA).

[c13] 13. The separating material of any of claims 1 to 12, wherein the polymerrizable monomers are selected from compounds of the following formula:
$H_2C=C(R^1)-C(O)-X-R^2-N(R^3)_2$,
wherein R^1= hydrogen, methyl or ethyl group; R^2= C1-C6-alkyl or aryl group; R^3= methyl or ethyl group; and X= NH or O.

[c14] 14. A method for the production of a separating material by:
providing a solid substrate, having amino-functional groups coupled to the substrate surface,

covalently coupling of the amino-functional groups with a thermally labile radical initiator,
contacting the substrate surface with a solution of polymerizable monomers under conditions, where thermally initiated graft copolymerization of the monomers takes place, to form a structure of adjacent functional polymer chains on the surface of the substrate.

[c15] 15. The method of claim 14, wherein the solid substrate is a porous polymeric material, preferably a porous polymeric material having a pore size that is sufficiently large to allow passage of blood, blood plasma, or blood serum through the substrate material.

[c16] 16. The method of any of claims 14 and 15, wherein the solid substrate is in the form of a membrane, a hollow fiber membrane, a particle bed, a fiber mat, or beads, preferably a hollow fiber membrane.

[c17] 17. The method of any of claims 14 to 16, wherein the solid substrate is made of a biocompatible material.

[c18] 18. The method of any of claims 14 to 17, wherein the solid substrate is made of a material selected from the group, consisting of polyacrylates, polystyrene, polyethylene oxide, cellulose, cellulose derivatives, polyethersulfone (PES), polypropylene (PP), polysulfone

(PSU), polymethylmethacrylate (PMMA), polycarbonate (PC), polyacrylonitrile (PAN), polyamide (PA), polytetrafluorethylene (PTFE), cellulose acetate (CA), regenerated celand blends or copolymers of the foregoing, or blends or copolymers with hydro-philizing polymers, preferably with polyvinylpyrollidone (PVP) or polyethyleneoxide (PEO).

[c19] 19. The method of any of claims 14 to 18, wherein the amino-functional groups are primary amino groups.

[c20] 20. The method of any of claims 14 to 19, wherein the thermally labile radical initiator, as the starting material before coupling to the amine groups on the substrate, comprises at least one, preferably two carboxylic groups.

[c21] 21. The method of any of claims 14 to 20, wherein the thermally labile radical initiator includes compounds which decompose to give free radicals on thermal activation, preferably the thermally labile radical initiator being selected among azo compounds or peroxides.

[c22] 22. The method of any of claims 14 to 21, wherein the thermally labile radical initiator is 4,4'-azobis-(4-cyanovaleric acid) or 2,2'-azobis-(N-(2-carboxyethyl)-2-methylpropionamide

.

[c23] 23. The method of any of claims 14 to 22, wherein the polymerizable monomers are selected from compounds having a polymerizable double bond.

[c24] 24. The method of any of claims 14 to 23, wherein the polymerizable monomers are selected from the group, consisting of acrylic acid, methacrylic acid, vinyl compounds, and derivatives of the foregoing compounds, N,N-Dimethylaminoethyl acrylamide, N,N-Diethylaminoethyl acrylamide, N,N-Dimethylaminopropyl acrylamide (DMPA), N,N-Dimethylaminopropyl methacrylamide, N,N-Dimethylaminoethyl methacrylate, N,N-Diethylaminoethyl methacrylate, N,N-Dimethylaminoethyl acrylate, N-Morpholinoethyl acrylate, N-Morpholinoethyl methacrylate, 1-Vinylimidazole, Trimethylammoniumethyl acrylamide, Trimethylammoniumpropyl methacrylamide, Trimethylammoniumethyl methacrylate, Glycidyl acrylate, Glycidyl methacrylate, Vinyl glycidyl ether, Vinyl glycidyl urethane, 2-Hydroxyethyl methacrylate, 2-Hydroxypropyl methacrylate, Hydroxymethyl methacrylate, N-Vinylpyrrolidone, 2-Vinyl pyridine, 4-Vinyl pyridine, N-Vinyl-2-methylimidazale.

[c25] 25. The method of any of claims 14 to 24, wherein the

polymerizable monomers comprise Dimethylaminopropyl acrylamide (DMPA).

[c26] 26. The method of any of claims 14 to 25, wherein the polymerizable monomers are selected from compounds of the following formula:
$H_2C=C(R^1)-C(O)-X-R^2-N(R^3)_2$,
wherein R^1= hydrogen, methyl or ethyl group; R^2= alkyl or aryl group; R^3= methyl or ethyl group; and X= NH or O.

[c27] 27. Use of a separating material of any of claims 1 to 13 for the extracorporeal treatment of blood, blood plasma or blood serum.

[c28] 28. The use of claim 27 for the extracorporeal removal of endotoxins from blood, plasma or serum of septic patients.

[c29] 29. Use of a separating material of any of claims 1 to 13 for affinity adsorption, ion-exchange adsorption, hydrophobic adsorption, hydrophilic adsorption, or affinity adsorption applications.

[c30] 30. A separating column comprising the separating material of any of claims 1 to 13, whereby the separating material is in the form of beads, the beads being packed into the column, and the beads having a size sufficient

to provide a porosity allowing passage of blood cells through the column.

[c31] 31. A separating cartridge, comprising a tube, multiple hollow fiber membranes potted into the tube, the tube being fitted with ports, and the membranes having a pore size sufficient to allow passage of blood plasma through the membrane, wherein the membrane is made of the separating material of any of claims 1 to 13.

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Material for Separating Components in A Biologic Fluid

Abstract

A material for separating the components of a fluid, particularly a biologic fluid such as blood. The material may have a solid substrate with functional amino groups coupled to a substrate surface. A thermally labile radical initiator may be covalently coupled to the amino-functional groups. The substrate surface may be contacted with a solution of polymerizable monomers under conditions where thermally initiated graft copolymerization of the monomers may occur, forming a structure of adjacent functional polymer chains on the surface of the substrate.

Fig. 1

Reaction scheme 1

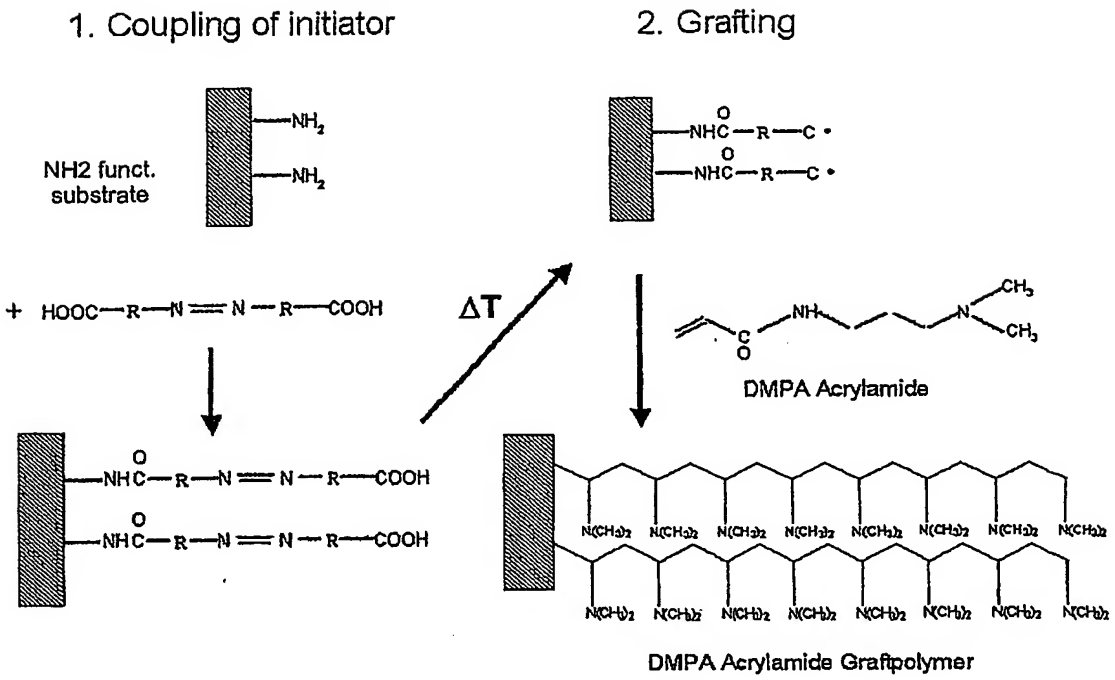


Fig. 2

Reaction scheme 2

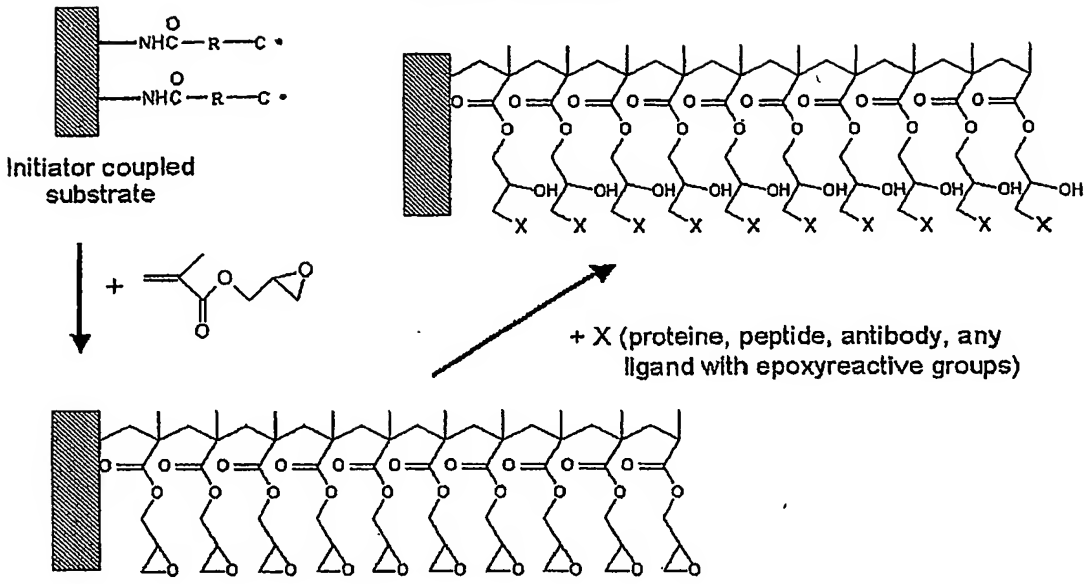


Fig. 3

Reaction scheme 3

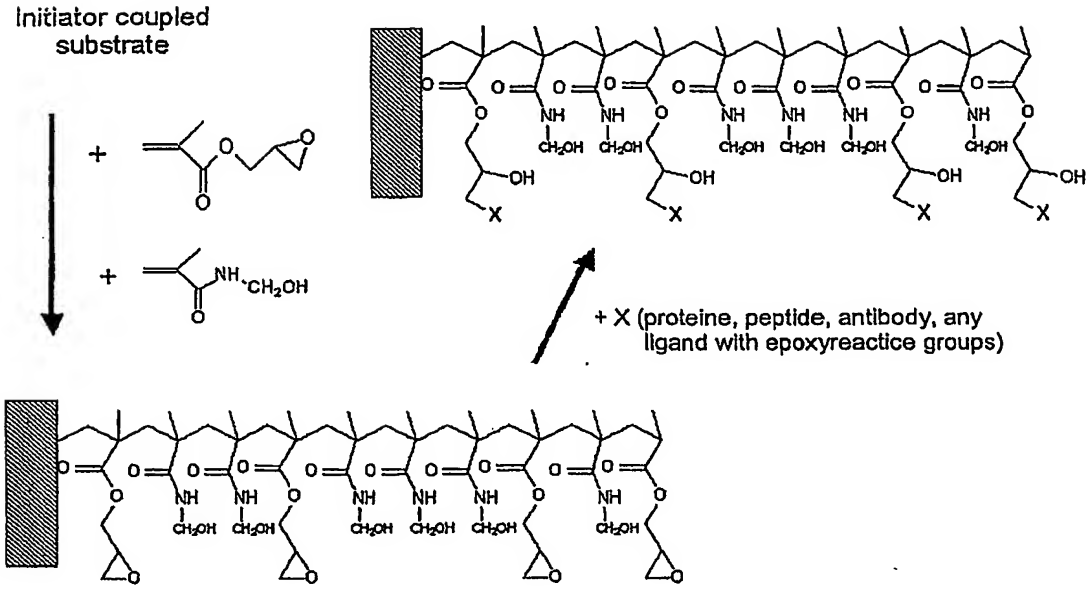


Fig. 4 **Endotoxin (LPS) concentration before and after columns (example 9)**

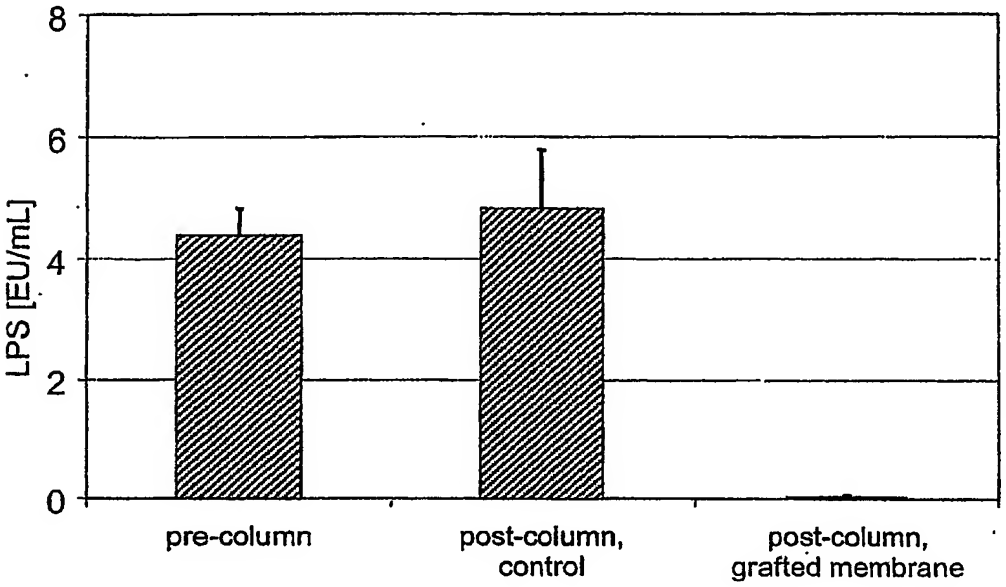
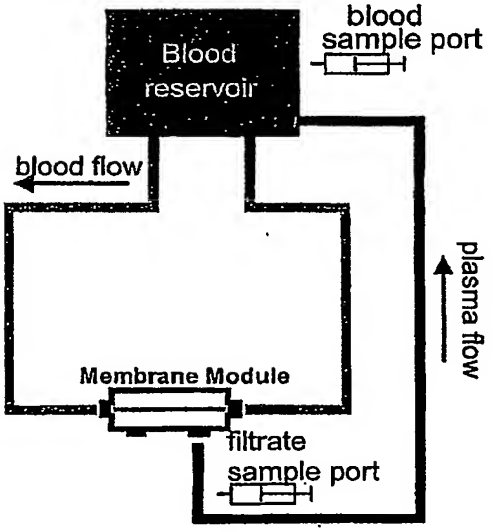


Fig. 5 **Experimental set-up for dynamic Endotoxin adsorption of grafted membranes from citrate-anticoagulated human blood (Example 10)**



- Blood from healthy donor, V=70ml , T=37°
- LPS: E. coli, 3 EU/ml
- 270 min recirculation
- Membrane module: A=200 cm^2, n(fiber)=144, l_{eff}=12 cm fibers
- $\gamma = 500s^{-1}$ (Q_B=8ml/min), Q_F=1ml/min, TMP<50mm_{Hg}

Fig. 6 Endotoxin reduction in filtrated plasma fraction (example 10)

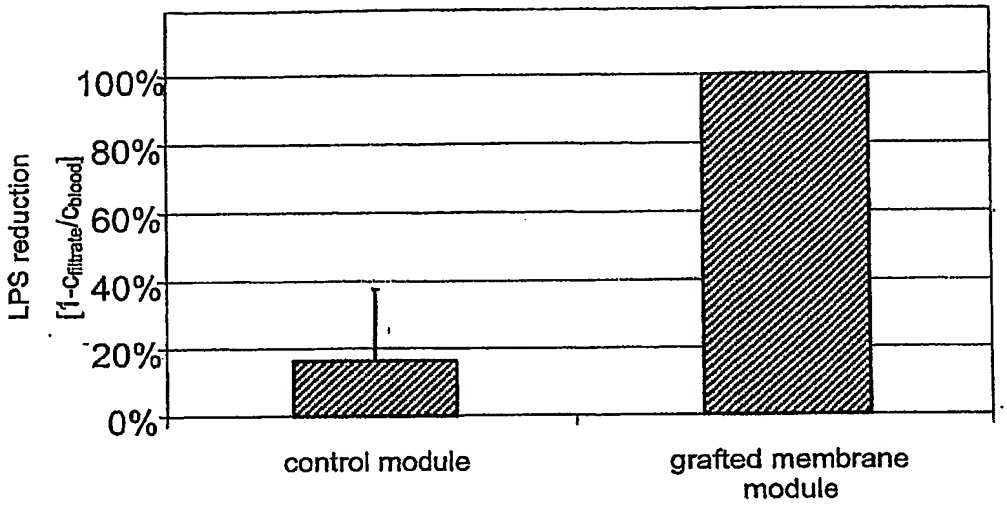


Fig. 7 Endotoxin concentration in blood reservoir (example 10)